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Flypaper for Parasites

In this issue, Kamhawi et al. (2004) describe the identification of an insect galectin as the receptor for the stage-specific *Leishmania* adhesin lipophosphoglycan (LPG). This interaction is critical for parasite survival in the midgut of its sand fly vector. The results open new avenues for studies of insect immunity, transmission binding vaccines, and host-parasite coevolution.

A key challenge for microbial pathogens is transmission—no matter how masterfully they survive in the face of host defenses, if further transmission isn't successful, they are evolutionarily terminated. Thus, transmission is often the "Weakest Link" of the infectious cycle, and most amenable to human interventions, as judged by the fact that some of the most successful and oldest methods block transmission (quarantines, destruction of reservoirs). For arthropod borne diseases transmission poses further challenges, as the pathogen must traffic between two hosts, each elaborating impressive arrays of antimicrobial defense mechanisms.

Kamhawi et al. (2004, this issue of *Cell*) now report on the identification of an insect molecule central to survival and transmission of the protozoan parasite *Leishmania* by their insect host, biting phlebotomine sand flies. *Leishmania* are responsible for a wide array of mild to fatal diseases, in tropical regions, developing countries, and the Mediterranean basin. Where transmission overlaps with HIV this parasite is a common opportunistic AIDS pathogen, and increasingly citizens in the developed world encounter these parasites. Often this arises through travel to endemic regions; for example, this has become a concern for foreign military personnel in Iraq. Additionally, leishmaniasis is increasingly being viewed as a cryptic, emerging disease, as reported recently in upper New York State in dogs (Enserink, 2000).

Like their trypanosome relatives, *Leishmania* reside within the digestive track of the fly until the next biting "opportunity". There they resist digestion and bind to the midgut epithelium, avoiding excretion as the digested blood meal passes from the fly (Figure 1). *Leishmania* bind through lectin-like interactions, often involving the polymorphic surface parasite glycolipid termed lipophosphoglycan (LPG; see Figure 1B in Kamhawi et al. for the structure). For the Friedlin V1 strain of *Leishmania major*, the backbone of the LPG "adhesin" is modified primarily with single galactosyl residues which mediate midgut binding, and parasites that show alterations in this structure fail to bind and are lost at the time of blood meal excretion (Sacks and Kamhawi, 2001).

Thus, the hunt was on for the sand fly LPG receptor. Through insightful studies characterizing genes expressed in sand fly midguts, a strong candidate emerged. PpGalec belongs to the large family of galectins, many of which are implicated in cellular signaling processes (Rabinovich et al., 2002). While the role(s) of insect galectins are largely unknown, the phylogenetic and cellular expression profiles of the PpGalec suggested it was the postulated sand fly receptor. This was confirmed by Kamhawi et al. (2004) in a series of elegant studies, first establishing that PpGalec was able to bind *Leishmania* mutants and strains as expected, and most importantly, that this association was critical for parasite survival. These studies mark the first example of a fly molecule critically implicated in *Leishmania* transmission. Even better, they provide answers and clues relevant to many questions holding great promise for future research.

For *Leishmania*, binding to the midgut receptor is a "double-edged sword": while critical to prevent excretion, the parasite must release when the insect feeds on the next susceptible animal. If binding dominates, "you may never return", like the legendary Charlie on the MTA. Thus, *Leishmania* orchestrates release from its midgut receptor to coincide with differentiation to the infective metacyclic stage (Figure 1A of Kamhawi et al.). At that time, the midgut binding form of LPG is shed, and replaced with a structurally modified LPG, unable to bind, but still able to carry out essential LPG functions needed later in the infectious cycle (Beverley and Turco, 1998). In *L. major* Friedlin V1, this is accomplished by "covering up" the galactose residues through the action of arabinosyl transferases (Figure 1; Dobson et al., 2003). Once freed from midgut binding, the parasite can be transmitted by the next bite, probably through regurgitation (Rogers et al., 2004). Notably, this problem of timing of adhesion and release is a general one, affecting transmission of many microbes other than protozoa.

Sand fly-*Leishmania* interactions show considerable strain and interspecific variation—both in terms of efficiency of survival and transmission, and the modifications of LPG mediating binding and subsequent release. Thus, one of the most exciting findings was that the PpGalec expression and gene is restricted only to species able to transmit *L. major* with monogalactosyl LPG modifications. Yet as one surveys the emerging diversity of LPG structures across *Leishmania* strains and species, there is great variation in stage- and species-specific LPG modifications, including poly-galactosyl, glucosyl, arabinosyl, mannosyl and/or mannosyl-phosphate modifications (Sacks and Kamhawi, 2001). This implies that there is a corresponding diversity of sand fly receptors to be discovered. Evidently, *Leishmania* and sand flies are undergoing a coevolutionary "arms race"; how this diversity in lectin and LPG is encoded and then read out from the genome of both players during evolution promises to be of considerable interest.

Why do sand flies elaborate the parasite receptor PpGalec? It seems unlikely that flies purposefully welcome parasites, as many researchers believe that *Leishmania* comes with a price to sand fly health. An exciting prospect may involve the typical properties of the galectin family, which often include signaling pathways (Rabi-

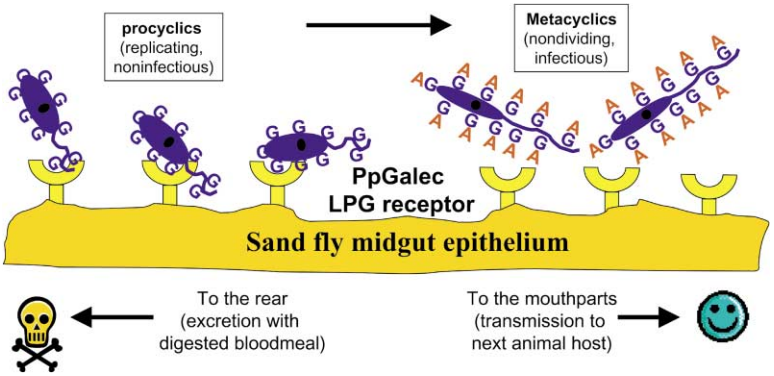

Figure 1. Overview of the Binding and Release of *Leishmania* to the PpGalec LPG Receptor during Parasite Development in the Sand Fly

Leishmania parasites (blue) reside normally in the midgut of Phlebotomine sand flies following acquisition through blood feeding. As the blood meal is digested, replicating, noninfective promastigote forms must bind to the midgut epithelium to avoid excretion. For the Friedlin strain of *Leishmania major*, this occurs by binding to the sand fly galectin receptor PpGalec identified by Kamhawi et al. (2004, this issue of *Cell*), which recognizes galactosyl modifications (G) of the abundant surface lipophosphoglycan coat (LPG, not shown; see Figure 1B of Kamhawi et al. for a complete structure). When parasites differentiate to the highly infective, non-dividing metacyclic stage, LPG is replaced with a modified version where the galactosyl residues are capped by arabinosyl modifications (A). This blocks binding to PpGalec and allows migration anteriorly for transmission.

novich et al., 2002). One could imagine a variety of interactions—perhaps PpGalec stimulates an "inhibitory" cascade leading to downregulation of insect defenses, or an "activating" cascade that contributes to a largely futile effort to rid itself of the offending parasite. LPG has been implicated in a variety of signaling interactions with host cells (Beverley and Turco, 1998), and it is tempting to speculate that the parasite has employed similar mechanisms in coping with the defenses of both hosts through its LPG surface coat. Notably, mammalian galectins have also been implicated in *Leishmania* interactions with vertebrate macrophages (Pelletier et al., 2003).

An important finding was that anti-PpGalec antibodies inhibited parasite midgut survival, which should mitigate transmission to the next host (this remains to be shown experimentally). This suggests the possibility of a transmission blocking vaccine against *Leishmania*. Such vaccines are an "altruistic" approach, aimed toward interrupting the infectious transmission cycle as the vaccinated host is not itself protected. However, leishmaniasis is primarily a zoonoses, where the transmission cycle is primarily animal-fly rather than human-fly. Many mammalian species serve as abundant natural hosts, and human immunizations would be expected to have little impact on transmission (it might also be difficult to explain to Congressional Committees why one is seeking to vaccinate Iraqi rodents). However, in some regions primary animal reservoirs may be amenable to vaccination (e.g., dogs), and in some settings, the parasite transmission cycle has evolved toward a primarily anthroponotic, human-fly cycle. There a transmission blocking vaccine might be successful.

Leishmania molecular genetics is well established, and many genes implicated in the biosynthesis of LPG and its stage-specific modifications are in hand. However, despite its importance to parasite biology and control, the number of laboratories focusing on the vector biology of *Leishmania* is small; sand fly colonies pose considerable difficulties to grow and maintain, and the flies could benefit from improved "domestication" as laboratory tools. Thus, the difficulty and challenge of these studies can't be overestimated. The success reported here shows that even a modest amount of genomic science coupled with good sand fly biology has paid off handsomely. Now proposals should be seriously considered toward determining the genome of these critical vectors, and the downstream development of functional genomic tools.

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